AD_____

Award Number: W81XWH-09-1-0460

TITLE: Imaging effects of neurotrophic factor genes on brain plasticity and repair in multiple sclerosis

PRINCIPAL INVESTIGATOR: Heather Wishart, Ph.D.

CONTRACTING ORGANIZATION: Dartmouth College
Hanover, NH 03755-1404

REPORT DATE: July 2012

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
July 2012	Annual	1 July 2011 – 30 June 2012

4. TITLE AND SUBTITLE

Imaging effects of neurotrophic factor genes on brain plasticity and repair in multiple sclerosis

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-09-1-0460

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Heather Wishart, Ph.D.

E-Mail: wishart@dartmouth.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Dartmouth College
Hanover, NH 03755-1404

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
The aim of this study is to develop a better biomarker for multiple sclerosis (MS) by combining genotype and imaging data. Patient with MS undergo evaluation to confirm diagnosis and determine disability level. They have blood drawn for genotyping, and undergo magnetic resonance imaging (MRI) sensitive to focal and diffuse changes in brain tissue (including cortical thickness and subcortical volume measures, lesion volumetry, and voxel-based morphometry and diffusion imaging). We continue to progress with screening, enrollment, and scanning of participants, with 101 patients having enrolled and completed study procedures or screened in to the study and in the process of scheduling. Although recruitment was initially slower than anticipated, we have taken major steps to increase visibility and awareness of the study among patients and referral sources, and our recruitment rate increased and continues to increase as a result. We continue to devote significant effort to maintaining and enhancing study visibility and awareness, and we fully anticipate that the momentum in recruitment will continue to grow as we go forward. We have encountered no adverse events. We continue to log and back up all data, and complete ongoing QA and data entry. A second batch of samples (N=46) has been submitted for TaqMan genotyping, which brings the total to N=93. We are preparing a preliminary report regarding Hypothesis 1-A. We are preparing an R01 application to study the imaging-genetics as a predictor of longitudinal outcomes in MS. Final findings from the research will be available when we complete data collection, at which time we will have sufficient statistical power for the analyses. We continue regular research meetings.

15. SUBJECT TERMS
Multiple sclerosis, MRI, genotype

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	5	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

Introduction

Background. Conventional MRI provides a useful biomarker for multiple sclerosis (MS) clinical trials, but conventional imaging is insensitive to many of the neural changes in the disease and a better biomarker is needed. The proposed study will combine multimodal MRI, to capture the full range of neural changes in the disease, with genotype data to develop a more sensitive and comprehensive biomarker for the disease. Neurotrophic factor genes related to brain plasticity and repair will be targeted for this purpose because of their probable moderating effects on neural damage in MS. Objective**.** The objective of the proposed study is to develop a better biomarker for MS by combining genotype and imaging data. Specific Aims**.** We will combine data regarding neurotrophic factor genotype and imaging to: (1) Assess specific *a priori* hypotheses regarding effects of specific neurotrophic factor polymorphisms on specific brain imaging measures, (2) Assess both additive and interactive models of effects of genes within each of the three neurotrophic factor gene families on imaging of gray and white matter integrity, and (3) Determine the optimal linear combination of genotype and imaging data to predict concurrent level of disability in MS. Study Design**.** Patients with MS (N=200) will undergo evaluation to confirm diagnosis and determine disability level. They will have blood drawn for genotyping, and will undergo MR imaging sensitive to both focal and diffuse effects in gray and white matter, including cortical thickness and subcortical volume measures, lesion volumetry, and voxel-based morphometry and diffusion imaging. Regression and symbolic modeling will be used to address the specific aims. A number of data reduction and other procedures will be used to minimize Type II error. Impact. This research will set the stage for future longitudinal research assessing the obtained imaging-genotype biomarker as a predictor of disease course and treatment response. Ultimately this research will improve the clinical care of patients with MS by increasing prognostic accuracy and enhancing our ability to identify optimal treatment protocols for individual patients. Development of a more sensitive and comprehensive biomarker will contribute to drug discovery and clinical trials in MS.

Body

We continue to progress with screening, enrollment, and testing. We have encountered no adverse events and our IRB/Human Subjects approvals are current. We had completed the following recruitment/ enrollment steps by June 30, 2012:

576 patients had completed basic screen or been referred to the study
361 passed basic screen and we had attempted/are attempting to contact for full screen
176 had completed full screen:
- 101 had enrolled and completed study procedures, or were in the process of scheduling
- additional 67 were currently in active recruitment (i.e., multistage screening for eligibility, MRI safety, etc.)

The funds for this study were released to us in October 2009, which is when we started recruiting. Recruitment was slower than anticipated in the early stages, but we have taken major steps to increase visibility and awareness of the study among patients and referral sources, and our recruitment rate has significantly increased as a result. In addition to advertizing through the MS Center at Dartmouth using study pamphlets and letters, we also regularly attend MS Center meetings on a biweekly basis, and the study coordinators in the MS Center are aware of the study and inform patients about it. We have also reached out to neurologists who see MS patients outside the regular clinic, as well as other departments (e.g., Radiology, Urology) where MS patients are seen at our medical center. We have made our study pamphlets available to the clinicians who see these patients and in the waiting areas. Our medical center's neuro-opthalmologist, who routinely sees patients with optic neuritis (who may be eligible for the study), is now also participating in referring to the study. We have also obtained the assistance of our hospital's patient education center, where study pamphlets are displayed, and we have had a number of self-referrals from individuals who saw our research advertized there. Our local MS Support Group meets in the patient education center, and they have the information available about the study. In addition to increasing visibility and awareness of the study throughout our medical center, we have also reached out to area MS Clinics in Lebanon, NH, Concord, NH, Manchester, NH, and Burlington, VT, and we have identified several additional clinics to include. We have study letters that are signed by each patient's MS neurologist to introduce the study to patients from these area clinics, as well as our own MS Center. In addition to recruiting through clinic and hospital settings, and support groups, we are also regularly attending regional MS patient-oriented conferences, where we have made presentations, and displayed our study materials, to increase visibility of the study, and these strategies have also been helpful in increasing recruitment. We have recently been able to fill our lab administrator position, and she is

working to update our website to facilitate recruitment into studies. Together, these efforts have paid off with significantly increased recruitment. We will continue to devote significant effort to maintaining and enhancing study visibility and awareness among referral sources and patients to maximize recruitment, and we fully anticipate that the momentum in recruitment will continue.

We continue to use our established procedures for acquiring data, including blood draw, testing, and MRI, and for logging and backing up all data. We complete ongoing QA, database entry, and preprocessing of image data as the data are acquired. We have completed genotyping on two batches of samples (N=46 and N=47), and present preliminary findings below. We are monitoring progress toward recruitment goals, and monitoring budget and expenses together with our grants manager and lab administrative director. We recently submitted our annual IRB renewal. We have encountered no adverse events.

Preliminary Data for Individuals with TaqMan Genotyping Completed

Background on Genetic Variations Tested to Date All participants have provided blood samples that we have submitted via our Phlebotomy Service to our Molecular Diagnostics Lab at Dartmouth-Hitchcock Medical Center/ Geisel School of Medicine at Dartmouth for storage and analysis. At the Molecular Diagnostics Lab, DNA is isolated and stored until use. Genotyping is done in batches to keep costs low and streamline the work. To date, two batches of samples (N=46 and N=47) have been submitted for analysis of selected key polymorphisms using TaqMan assays. We present preliminary results in relation to imaging and testing outcomes for these participants. The genome-wide association study (GWAS) testing will await collection of all 200 samples, because of minimum sample size requirements for this type of testing.

Key genes from the neurotrophin and growth factor family selected for TaqMan analysis included the brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and the BDNF receptor NTRK2, both of which are expressed in MS lesions and appear to play a neuroprotective role.[1-3] Also tested were human leukocyte antigen (HLA) DRB1*1501, which is a major risk locus for MS,[4, 5] and apolipoprotein E (APOE), because of its role in brain response to brain injury.[6] Of the 93 samples submitted for these TaqMan asssays, 89 produced valid results for these four key genetic variations on the first testing, and four are being re-evaluated in the Molecular Diagnostics Lab. Also tested to date were a vitamin D receptor (VDR) polymorphism and, in a smaller subsample to date (N=58), neurotrophic factor 3 (NTF3) and nerve growth factor beta (NGFB) and the receptor NTRK3 and the ciliary neurotrophic factor (CNTFR), all members of the neurotrophic factor family of genes, which are the focus of this study. These are not all the genes that will be tested in our biological pathway of interest, but they are the focus of this report and, as noted above, additional genotype data will be available once we complete sample collection and genome-wide testing.

Participants Participant characteristics are presented in ***Table 1*** for the N=89 participants who have genotyping completed to date for BDNF, NTRK2, DRB1*1501, and APOE. The sample is predominantly female, as expected for this clinical population (68 females, 21 males), and includes patients with relapsing-remitting MS (n=76), secondary progressive disease (n=12), and clinically isolated syndrome (n=1).[7, 8] Age at diagnosis was available in all cases (Table 1), but age at symptom onset could not be ascertained reliably in four patients. Available data indicated that patients were experiencing mild to moderate disability, as measured using the Expanded Disability Status Scale. The MS Functional Composite serves as an additional, complementary measure of MS disability, and is composed of standard tests of cognition and upper and lower extremity motor function. Relative to the Composite's normative database of MS patients, available data on this measure indicated that individuals in this sample were in the average range of disability for patients with MS.

Table 1. *Participant Characteristics.*

	N	Mean	SD
Age (years)	89	47.3	10.2
Education (years)	89	15.2	2.6
Age at first MS Symptom (years)	85	32.9	9.3
Age at MS Diagnosis (years)	89	39.1	9.7
Expanded Disability Status Scale (EDSS)	83	2.4	1.7
MS Functional Composite (z-score)	82	0.2	0.6

Procedures

Image Acquisition. MR images are acquired on our research-dedicated Philips Achieva 3T scanner with 8-channel head coil. Using our standard procedures, participants are situated in the scanner with earplugs and headphones to attenuate sound and help immobilize the head, and instructed to keep their head and the rest of their body as still as possible. Motion parameters are recorded for use in subsequent QA and preprocessing. Structural images include a sagittal survey 3-plane localizer, an M2D/TFE T1-weighted survey with 10mm slice thickness; a sagittal T1-weighted MP-RAGE 3D anatomical volume (170 contiguous 1.2 mm sagittal slices, TR: 6.8ms, TE: 3.2ms, TI: 852.9ms, TFE prepulse delay: shortest, flip angle: 8 deg, NEX: 1, BW/Pixel: 241, FOV: 256mm, matrix 256x256, 1.0 mm^2 in-plane resolution); an axial fluid-attenuated inversion recovery (FLAIR) scan (slice thickness: 3mm, TR: 11000, TE: 125, TI: 2800, TFE: 27, flip angle: 120, NEX: 1, BW/Pixel: 223.9/1.940, FOV: 240 mm); and a diffusion tensor image (DTI; 46 diffusion directions, 65 2mm transverse slices, FOV 256; TR: min.; TE: 76, FA: 90, Matrix: 128).

Image Processing.

(i) FLAIR Lesion Segmentation. The FLAIR scans are segmented for total lesion volume using a semi-automated approach we have developed and validated for this purpose.[9, 10] Using an expectation-maximization algorithm, the program ("FLAIRSEG") classifies each voxel in the image as lesion, normal tissue, or cerebrospinal fluid. The resulting lesions masks are edited manually to ensure correct voxel classification, a process that takes approximately 10 minutes per brain. We have previously validated FLAIRSEG results against our "gold standard" manual lesion segmentations (intraclass coefficient = 0.94, $p < .001$), which were performed by an experienced rater together with either Heather Wishart (PI) or Alexander Mamourian, MD (a Board-certified neuroradiologist).[9] Inter- and intra-rater reliabilities for total lesion volume, assessed using the intraclass correlation coefficient, are 0.94 or higher.[9, 10]

(ii) T1 Hypointense Lesion Segmentation. Following segmentation of the FLAIR hyperintense lesions, the FLAIR scans and FLAIR lesion masks are then registered to the MPRAGE for segmentation of T1 hypointense lesions. Using an initial automated approach, each T1 voxel that falls within the registered FLAIR lesion mask is classified according to whether or not it meets a predetermined intensity threshold, and the resulting masks are manually edited by a trained image analyst.

(iii) T1 Volume Segmentation and Analysis. T1 volumes are processed and segmented for cortical and subcortical volumes using FreeSurfer[11] (http://surfer.nmr.mgh.harvard.edu/fswiki/FreeSurferWiki). In brief, the processing steps include removal of nonbrain tissue in the images, registration to Talairach space, segmentation of subcortical structures based on *a priori* maps of these regions, and intensity gradient-based delineation of gray/white/CSF boundaries. These processes yield volumes for gray, white and CSF compartments, as well as subcortical volumes (e.g., hippocampus, thalamus). Further processing yields cortical thickness estimates. FreeSurfer has been used and validated in prior MS studies [12, 13], and has been shown to be reliable across different types and field strengths of scanners (http://surfer.nmr.mgh.harvard.edu/fswiki/FreeSurferWiki).

(iv) Voxel-based morphometry and diffusion tensor imaging analyses are also being completed as part of the study.

Blood Draw and Genotyping. Genetic polymorphisms are analyzed from patient DNA isolated from peripheral blood samples using the Gentra Puregene Blood Kit (Qiagen; Valenica, CA). In the case of APOE, two single nucleotide polymorphisms (SNPs; 334 T/C and 472 C/T) are examined, as previously described [14]. The APOE allotype is determined by the combination of SNPs at either one or both of these positions (334 and 472). The remaining polymorphisms are genotyped using pre-designed allele-specific primer and probes from Applied Biosystems (Foster City, CA). Applied Biosystems TaqMan® SNP Genotyping Assays contain a pair of polymerase chain reaction oligonucleotide primers flanking the region of interest and one wild-type oligonucleotide probe fluorescently labeled with VIC and another probe for the mutation labeled with FAM. All primers and probes are used at final concentrations of 900 nM and 200 nM, respectively in 1X TaqMan® Universal PCR Master Mix (Applied Biosystems; Foster City, CA) with 5-20 ng of genomic DNA on a 7500 Fast Real-Time PCR System using allelic discrimination analysis with the 7500 Software v2.0 (Applied Biosystems).

Preliminary Results

Genotyping. Data for the individual genotype assays to date are presented in ***Table 2***. The focus of analysis to date has been on BDNF, NTRK2, DRB1*1501, and APOE genotypes, and additional genotypes are in process. With the exception of DRB1*1501, the results are in Hardy-Weinberg equilibrium (HWE). DRB1*1501 is not in HWE, which is as expected given its disease association (***Table 2***). These data indicate that the genotype results for the sample conform to expected population distributions.

Table 2. *Genotype Distributions*

BDNF, NTRK2, DRB1*1501 Genotypes								
		N	Common Homozygotes	Heterozygotes	Rare Homozygotes	Chi-Squared	P Value	HWE
BDNF	rs6265	89	55	32	2	1.16	> 0.05	Yes
NTRK2	rs1187323	89	63	25	1	0.84	> 0.05	Yes
DRB1*1501	rs3135388	89	46	43	0	9.03	< 0.005	No

APOE Genotype											
		N	ε2/ε2	ε2/ε3	ε3/ε3	ε2/ε4	ε3/ε4	ε4/ε4	Chi-Squared	P Value	HWE
APOE	rs429358 & rs7412	89	1	16	49	1	17	5	5.8	> 0.05	Yes

Additional TaqMan Results								
		N	Common Homozygotes	Heterozygotes	Rare Homozygotes	Chi-Squared	P Value	HWE
NGFB	rs6330	57	25	22	10	1.66	> 0.05	Yes
VDR	rs731236	86	37	41	8	0.49	> 0.05	Yes
NTF3	rs6332	58	18	30	10	0.17	> 0.05	Yes
NTRK3	rs999905	58	24	24	10	0.85	> 0.05	Yes
CNTFR	rs7036351	58	39	16	3	0.61	> 0.05	Yes

Image Data. Total volumes for the gray and white matter compartments, as well as intracranial volume, are presented in **Table 3**, along with thalamic, hippocampal, and FLAIR lesion volumes. For the purpose of statistical analyses, gray matter fraction is calculated as the volume of the total volume of the gray matter compartment over the sum of the gray, white and CSF compartments, multiplied by 100. White matter fraction and brain parenchymal fraction are calculated similarly. Thalamic and hippocampal volumes are normalized relative to total gray and white matter volume. Values were not yet available for two patients for FreeSurfer volumes. Additional FLAIR lesion volumes are in processing.

Table 3. *Brain Volumes (mm^3)*

	N	Mean	SD
Gray Matter Volume	87	563,699.9	61,040.8
White Matter Volume	87	477,160.3	65,609.2
Intracranial Volume	87	1,235,766.9	202,197.5
Left Thalamus	87	6292.6	975.2
Right Thalamus	87	6158.2	928.7
Left Hippocampus	87	3985.5	457.8
Right Hippocampus	87	4036.3	548.0

FLAIR Lesion Volume	72	12,525.7	24,613.9

Genotype-Imaging Relationships.

These preliminary analyses focus on BDNF, NTRK2, DRB1*1501 and APOE genotypes in relation to imaging and disease variables. As expected, these genotypes were not correlated with each other (all $p > 0.05$).

Simple correlations revealed no simple relationship between BDNF or NTRK2 genotype and normalized hippocampal and thalamic volumes ($p > 0.05$), and this pattern of findings remained the same after covarying for APOE and DRB1*1501 genotype. This suggests no single gene effects for either BDNF or NTRK2 (its receptor) on volumes of these *a priori* regions of interest in this sample size. In AIM I, we will test for additional single gene effects using voxel-based imaging approaches.

Aim II is based on the idea that, given the complexity of a disease such as MS, it may be more likely that multiple additive or interacting gene effects influence disease course, rather than single gene effects. As a preliminary analysis related to this aim, an analysis of covariance examining the interaction of BDNF and NTRK2 genotype was conducted with gray matter fraction as the dependent variable, covarying for effects of DRB1*1501 and APOE genotype. The F value for the interaction effect was 2.0 ($p = 0.16$). This effect is not statistically significant with this sample size, but suggests the promise of the approach in Aim II, and we will follow up in the complete sample in which we will have sufficient statistical power to adequately test these effects.

As a preliminary analysis related to Aim III, to test for the best predictors of MS disability, a regression analysis was conducted with genotype (BDNF, NTRK2, APOE, DRB1*1501), brain parenchymal fraction, and gender as predictors of disability as measured using the Expanded Disability Status Scale scores, adjusted for duration of disease since symptom onset. Results approached significance for the full model ($F(6, 70) = 1.94$, $p = 0.086$). This effect appeared to be due mainly to effects of NTRK2 and APOE genotype ($p = 0.017$ and 0.052 respectively). While this analysis is preliminary and does not assess the full model proposed in Aim III, the results lend general support to a multimodal approach combining imaging, genotype and other data to predict disability progression in MS.

Key Research Accomplishments

- This study's final key findings will be reportable when we have completed data collection. Preliminary analyses suggest that:
    - The genotypes assessed in this study are in Hardy-Weinberg equilibrium, with the exception of DRB1*1501, which is as expected given its disease-association. This suggests that the sample genotype characteristics conform to expected population distributions.
    - As might be expected given the complexity of MS, single gene effects on disease progression, as measured using imaging, may be small to negligible. This remains to be fully tested in the complete sample.
    - An approach assessing multiple small additive or interacting genotype effects may be more fruitful. In this sample, the interaction effect for BDNF and NTRK2 (its receptor) on brain parenchymal fraction appeared greater than the effect of either polymorphism alone.
    - By combining information from imaging, genotype, and other variables, we may be able to determine the best predictors of disability, and more importantly progression of disability, in this disease. In this preliminary analysis, results for a model combining genotype, imaging and gender suggested that the combined approach accounted for a proportion of variance that approached significance in this sample size. We will have final results when the complete sample is available.

Reportable Outcomes

- One grant application was awarded to add a cognitive testing component to this study (National MS Society RG4264A3)
- We are preparing an R01 grant application to perform a longitudinal follow-up of patients enrolled in this study.

Conclusion

This study aims to improve scientific understanding of the neural and genetic basis of individual differences in MS progression, identify protective and risk factors, and inform and develop an integrated imaging genetics approach for use as a biomarker in MS. The perspective of this study is that genes related to plasticity and repair may code for factors that limit the extent of demyelination and axonal injury in MS, thereby contributing to better overall disease course. Identifying important biological pathways related to resilience to MS could inform development of novel treatment approaches. Additionally, having a biomarker that could, from the beginning of the disease, help predict course, would greatly advance accuracy of prognostication and individualized treatment selection. By matching patients with the most appropriate and least invasive treatments early in the course of the illness, this would contribute to improved care for individuals with the disease and improved overall efficiency of delivery of health care for MS.

Our preliminary analyses to date suggest that an approach examining gene-gene interaction effects holds promise in developing an imaging biomarker to aid in achieving these goals. As might be expected given the complexity of this disease, it seems most probable that it is the cumulative load ("additive model") or multiple interacting effects ("interaction model") of these genotype variations that influences extent of neural changes. Additionally, the combination of genotype, imaging, disease, and demographic variables may lead to the best overall prognostic accuracy, as compared to approaches to date which have focused largely on individual variables in relative isolation. In a separate study, we have also obtained preliminary evidence of an interaction of DRB1*1501 genotype and smoking as predictive of MS disease progression. Going forward, we plan to continue to code for such environmental factors, broadly defined, to incorporate in our analyses.

Overall, this study will evaluate the hypothesis that individuals with a genotype pattern that favors brain plasticity and repair will have less overall extent of neural damage, as measured on imaging, and less accumulation of clinical disability. If so, this will open new windows on factors affecting disease progression, prognostication, and treatment selection in MS.

References – References cited are from the published literature. (They are not papers emanating from this study.)

1. Stadelmann, C., et al., *BDNF and gp145trkB in multiple sclerosis brain lesions: neuroprotective interactions between immune and neuronal cells?* Brain, 2002. **125**(Pt 1): p. 75-85.
2. Kerschensteiner, M., et al., *Activated human T cells, B cells, and monocytes produce brain-derived neurotrophic factor in vitro and in inflammatory brain lesions: a neuroprotective role of inflammation?* Journal of Experimental Medicine, 1999. **189**(5): p. 865-70.
3. Hohlfeld, R., et al., *The neuroprotective effect of inflammation: implications for the therapy of multiple sclerosis.* Neurological Sciences, 2006. **27 Suppl 1**: p. S1-7.
4. Sawcer, S., et al., *A high-density screen for linkage in multiple sclerosis.* American Journal of Human Genetics, 2005. **77**(3): p. 454-67.
5. Baranzini, S.E. and D. Nickles, *Genetics of multiple sclerosis: swimming in an ocean of data.* Current Opinion in Neurology, 2012. **25**(3): p. 239-45.
6. Waters, R.J. and J.A. Nicoll, *Genetic influences on outcome following acute neurological insults.* Current Opinion in Critical Care, 2005. **11**(2): p. 105-10.
7. McDonald, W.I., et al., *Recommended diagnostic criteria for multiple sclerosis: Guidelines from the International Panel on the Diagnosis of Multiple Sclerosis.* Annals of Neurology, 2001. **50**: p. 121-127.
8. Lublin, F.D. and S.C. Reingold, *Defining the clinical course of multiple sclerosis: Results of an international survey.* Neurology, 1996. **46**: p. 907-911.
9. Wishart, H., et al., *A novel approach for semi-automated segmentation of MS lesions on FLAIR imaging: Reliability and clinical correlates*, in *Annual Meeting of the International Neuropsychological Society*. 2010: Acapulco, Mexico.
10. Kee, E.R., et al., *Development and testing of a new approach to MS lesion segmentation using 3T FLAIR images*, in *Annual Meeting of the International Neuropsychological Society*. 2011: Boston, MA.
11. Fischl, B., et al., *Whole Brain Segmentation: Automated Labeling of Neuroanatomical Structures in the Human Brain.* Neuron, 2002. **33**: p. 341-355.
12. Sailer, M., et al., *Focal thinning of the cerebral cortex in multiple sclerosis.* Brain, 2003. **126**(Pt 8): p. 1734-1744.

13. Derakhshan, M., et al., *Evaluation of automated techniques for the quantification of grey matter atrophy in patients with multiple sclerosis.* Neuroimage, 2010. **52**(4): p. 1261-7.
14. Koch, W., et al., *TaqMan systems for genotyping of disease-related polymorphisms present in the gene encoding apolipoprotein E.* Clinical Chemistry & Laboratory Medicine, 2002. **40**(11): p. 1123-31.

Appendices NA

Supporting Data NA